Enhanced COMP catabolism detected in serum of animal models and OA patients through a novel capture ELISA

Enhancement of COMP fragment levels in serum of OA patients

Since mAb 2127F5 was found to recognize both murine and human COMP fragments, we also examined serum levels of the 2127F5 antigen in OA patients and controls blood samples. In parallel, whole COMP serum levels were also measured using a commercial COMP ELISA kit (Biovendor). As shown in Fig. 2B, no significant difference in total COMP levels was detected between symptomatic knee OA (SKOA) and non-OA control groups, while a significant increase of the 2127F5 COMP fragment was noted in the serum of SKOA patients when compared to that of non-OA controls.

In summary, using recombinant COMP fragments we have generated a series of mAbs recognizes diverse regions of the molecule and one of these antibodies reactive with the C-terminal portion of the molecule was further exploited to establish a unique sandwich ELISA capable of reproducibly measure the levels of COMP fragments (COMP catabolism) in the body fluids of murine OA models and OA patients. Thus, this system provides a valuable means to exploit COMP fragments biomarker for monitoring the effects of interventions.

Methods:
Expression and purification of recombinant COMP fragments; Generation of a panel of monoclonal antibodies (mAbs) against COMP fragments; immunochemical characterization of monoclonal antibody specificity; development of a capture ELISA based upon the fragment-specific mAbs; Statistic analyses.

Results and discussion:
Characterization of a mAb that specifically recognizes a COMP proteolytic fragment in OA: We have generated a panel of murine mAbs against recombinant COMP fragments and have identified one of these clones, 2127F5, as a mAb preferentially recognizing the COMP C-terminal domain. Whole serum from OA patients was precipitated with 100% TCA, separated by SDS-PAGE under reducing conditions and immunoblotted with either a polyclonal anti-COMP antiserum or mAb 2127F5. As shown in Fig. 1A, B, the antiserum recognized both intact COMP and its degradation fragments, whereas mAb 2127F5 specifically reacted with one COMP fragment with the apparent Mr of 55 kDa.

COMP fragments are significantly more abundant in serum of mice with collagen-induced arthritis (CIA): A sandwich ELISA was then designed to detect COMP and degradation products in serum of healthy and disease individuals. The anti-COMP antiserum coated onto ELISA plates was used as the capturing antibody, while mAb 2127F5 was used as a detection agent in conjunction with a secondary anti-mouse HRP-conjugated antibody. Serially diluted recombinant Type III domain fragments of COMP were used as standards. The analytical limit of detection was established to be roughly 0.2ng/ml. As a first screening with assessed the levels of COMP fragments in the serum of mice with CIA – a mouse model of human rheumatoid arthritis. As shown in Fig. 2A, circulating proteolytic fragments of COMP are significantly higher than those in control mice. This result suggests that the established ELISA can be exploited to detect altered levels of COMP catabolism in CIA murine model.

Fig. 1. Western-blotting assay of OA serum with polyclonal (A) or monoclonal antibody 2127F5B6 (B).

Fig. 2. (A) Relative serum levels of COMP fragments in healthy (CTR, n=8) and CIA mice (n=6). (B) Comparison between whole COMP serum levels assessed with a commercial ELISA kit and COMP fragment levels detected with the newly established COMP ELISA in healthy donors and OA patients. Values are shown as mean ± SEM. **p<0.02.

References:

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